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Gene Expression and Enzyme Activities of the Sodium Pump During Sea Urchin Development: Implications for Indices of Physiological State

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Abstract. The sodium pump consumes a large portion of the metabolic energy (40%) in sea urchin larvae. Understanding the developmental regulation of ion pumps is important for assessing the physiological state of embryos and larvae. We sequenced a partial cDNA clone (1769 bp) from the sea urchin *Strongylocentrotus purpuratus* and found it to contain the C-terminal portion of an open reading frame coding for 195 amino acids that exhibited high sequence similarity (89%) to invertebrate α-subunits of the Na^+,K^+-ATPase sodium pump. Northern blots using the 3′ untranslated region of this cDNA specifically recognized a 4.6-kbp transcript under high stringency. During embryonic development, a rapid increase in levels of this mRNA transcript during gastrulation (25 h postfertilization) was paralleled by a concomitant increase in the total enzymatic activity of Na^+,K^+-ATPase. Expression of this subunit during gastrulation increased to a maximum at 36 h, followed by a rapid decline to trace levels by 60 h. The rate of removal of the transcript from the total RNA pool after 36 h closely followed a first-order exponential decay model ($r^2 = 0.988$), equivalent to a degradation rate of 7.8% h^{-1}. By 83 h, transcription of the α-subunit gene was low, yet sodium pump activity remained high. Molecular assays for the expression of this gene would underestimate sodium pump activities for assessing physiological state because of the temporal separation between maximal gene expression in a gastrula and maximal enzyme activities in the later larval stage. This finding illustrates the difficulty of using molecular probes for assessing the physiological state of invertebrate larvae.

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Introduction

Maintaining Na^+ and K^+ ion gradients is one of the most energetically demanding processes of an organism's maintenance physiology. In general, animal cells routinely expend 20%–30% of their total metabolic energy on the activity of a single protein complex, the sodium pump (Na^+,K^+-ATPase; Siems *et al.*, 1982, 1992), and for adult marine invertebrates, the sodium pump can potentially account for 30%-70% of tissue metabolism (Baker and Connelly, 1966; Lucu and Pavicic, 1995). The ion gradients established by the sodium pump are critical for maintaining a cell's osmotic balance and resting membrane potential, as well as providing the electrochemical gradient necessary for the uptake of other ions, sugars, amino acids, and neurotransmitters *via* Na^+ coupled co-transporters (Blanco and Mercer, 1998).

The requirements for ion regulation change rapidly during embryonic development. The increase in cell number during early embryogenesis and the consequent increase in cellular-membrane surface area necessitates the production of more sodium pumps to regulate intracellular ion flux. The *in vivo* physiological activity of Na^+,K^+-ATPase has been characterized during early development in the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus* (Leong and Manahan, 1997). Using $^{86}Rb^+$ as a radioactive tracer for K^+ ion transport, Leong and Manahan (1997) described the ontogenetic changes in activity of

Na^+,K^+-ATPase in living embryos. They found a large increase in activity—from nondetectable levels prior to fertilization to a high level accounting for 40% of total metabolic energy consumption at the pluteus larval stage (72 h postfertilization). By the same radiotracer techniques, the metabolic energy demand of Na^+,K^+-ATPase activity in the Antarctic sea urchin *Sterechinus neumayeri* was found to be as high as 80% of total metabolism at the pluteus larval stage at −1.5°C (Leong and Manahan, 1999). In the sea urchin *Hemicentrotus pulcherrimus*, the total protein activity and gene expression of Na^+,K^+-ATPase increases rapidly during gastrulation (Mitsunaga-Nakatsubo *et al.*, 1992a, b). Overall, the physiological importance of Na^+,K^+-ATPase activity during embryogenesis in sea urchins has significant implications for metabolic energy consumption during development.

This universal importance of Na^+,K^+-ATPase in animals suggests that measurements of this enzyme could be a useful indicator of physiological state. For larval stages in which direct enzyme assays are limited by the small amount of protein in an individual, measurements of gene expression might provide the sensitivity necessary to assay small amounts of tissue. Functional Na^+,K^+-ATPase pumps are a heterodimer (α, β subunits; Jorgensen and Skou, 1969), with the α-subunit possessing the ATP binding site and catalytic activity (Kyte, 1971). In this study, we describe the timing between transcription of the α-subunit and the appearance of functional sodium pumps during the development of *Strongylocentrotus purpuratus*. We also describe the ontogenetic changes in expression of the α-subunit to determine the developmental timing between increases in enzyme activity and the potential for using these measures as an index of physiological state in embryos and larvae.

Materials and Methods

Embryo cultures

Adult *Strongylocentrotus purpuratus* were induced to release gametes (injections of 0.5 *M* KCl), and fertilized eggs were divided into six 20-liter culture containers at a concentration of about 20 individuals per milliliter of filtered seawater (0.2 μm). Culture temperatures were maintained at 15°C during development. Embryos were maintained in suspension by paddles connected to slow stirring motors (~30 rpm). For the gene expression analysis, time-course samples were collected throughout development at the following times from an egg to a 4-arm pluteus larva: 0, 6, 8, 10, 12, 14, 16, 18, 20, 25, 31, 36, 42, 48, 60, 72, and 83 h postfertilization (n = 17). For each sample, about 100,000 embryos were removed by sieving (80-μm mesh) and pelleted by centrifugation (1000 × g) into 50-ml screw-cap tubes. Embryos were immediately dissolved in an acid-guanidinium buffer (4 *M* guanidinium isothiocyanate, 25 m*M* Na-citrate, 0.2% Sarkosyl and 215 m*M* β-mercaptoethanol; pH 5.2; Chomzinsky and Sacchi, 1987) and frozen at −80°C.

cDNA clone: sequencing and analysis

An expressed sequence tag (EST) library from activated coelomocytes of adult *S. purpuratus* was prepared by Smith *et al.* (1996), and a sequence fragment of one cDNA clone (#020) was found to have a high nucleotide similarity to the bovine α-subunit of Na^+,K^+-ATPase. We sequenced this clone (provided by C. L. Smith and E. H. Davidson) by random transposon insertion in a modified pBluescript (Stratagene) plasmid (pMOB; Strathmann *et al.*, 1991). The introduced transposon elements contained defined priming sites for subsequent manual sequencing of double-stranded plasmid templates using standard dideoxy termination reactions with ^{35}S-labeled dATP (Sequenase Reaction Kit, USB). Sequencing gels were visualized by autoradiography on X-ray film (Kodak, XAR 5). Nucleotide sequences were entered and edited using the software package MacVector 5.0 (Mac OS; Oxford Molecular Group), and contiguous overlaps between fragments were identified using the software package AssemblyLign 2.0 (Mac OS; Oxford Molecular Group). Both strands of the open reading frame (ORF) were sequenced by overlapping subclones so that most of the contiguous ORF sequence was assembled from three independent sequencing reactions. For phylogenetic comparisons, nucelotide and putative amino acid sequences from other animal species were structurally analyzed and aligned using the OMIGA 2.0 software package (Oxford Molecular Ltd.). Identity and similarity scores for the deduced amino acid alignments were calculated from the FASTA routine available in the GCG Wisconsin Package 8.0 (UNIX OS).

mRNA analysis: isolation and quantification

Total RNA was extracted from each sample by an acid guanidinium-phenol method (after Chomzinsky and Sacchi, 1987) and further purified by sequential precipitations in lithium chloride (4 *M* LiCl), sodium acetate (3 *M* NaOAc, pH 4.2) and ethanol (70% EtOH). After each precipitation, the RNA pellets were washed in 70% EtOH and dried under vacuum; before proceeding with the next precipitation, the pellets were resuspended in RNase-free TEN buffer (10 m*M* Tris pH 8.0, 1 m*M* EDTA and 10 m*M* NaCl). The final RNA precipitates were resuspended in RNase-free water and quantified by their optical density at 260 nm. From each developmental time point, 10 μg of total RNA was size-separated by formaldehyde gel electrophoresis and blotted overnight *via* capillary transfer onto nylon membranes. RNA on the nylon membranes was UV cross-linked (Stratalinker), and the membranes were stored dry at room

temperature. A cDNA probe was generated from the 3′-untranslated region (UTR) of clone #020. The terminal 1185 bp were PCR amplified (5′- TGG GAT TGA AGG AGT CAG –3′ and T7 oligonucleotide primers) and gel purified for further use in standard Northern hybridizations (see general methods in Ausubel *et al.*, 1992). Membranes were prehybridized for several hours in 40% formamide, 25 m*M* Na_3PO_4 (pH 7.2), 5× SSC, 0.1% SDS, 5× Denhardt's, and 50 μg/ml yeast RNA at 45°C in a hybridization oven. The 3′-UTR PCR probe (1185 bp) was radiolabeled by random priming (Promega) with α-^{32}P-dCTP (3000 Ci mmol^{-1}), added to the hybridization tube with a fresh 10-ml aliquot of hybridization buffer (as above), and incubated overnight at 50°C. The blots were initially washed with 0.1× SSC, 1.0% SDS, and 0.5% $Na_4P_2O_7$ at 45°C for 1 h. Additional washes at higher temperatures (max. 55°C) were performed as necessary to further reduce the background signal. Autoradiograms (Kodak Biomax X-ray film) were digitized on a high-resolution scanner (1200 dpi), and grain densities for the signal bands were quantified using the image analysis routines in the software program PhotoShop 4.0 (Win95 OS; Adobe).

Na^+,K^+-ATPase enzyme activity

Total enzyme activity of Na^+,K^+-ATPase was measured at short intervals between 20 and 50 h postfertilization, the period during which enzyme activity increases rapidly during development in *S. purpuratus* (Leong and Manahan, 1997). Ouabain-sensitive Na^+,K^+-ATPase activity (details in Leong and Manahan, 1997) was determined in all samples on the same day with one set of standards to minimize the between-sample assay error. Total Na^+,K^+-ATPase activity was measured as the rate of hydrolysis of ATP (Esmann, 1988). Briefly, embryo tissues were thawed, sonicated, and resuspended in histidine buffer (10% sucrose, 5 m*M* EDTA and 5 m*M* histidine, pH 7.7) at a final protein concentration of 0.5 to 1.0 mg ml^{-1}. In the present study, the total Na^+,K^+-ATPase activity of the sea urchin embryos was measured as the difference in ATPase activity in the presence and absence of 2 m*M* ouabain at 25°C. A detailed consideration of the inclusion of detergents in the Na^+,K^+-ATPase assay is presented in Leong and Manahan (1997). In summary, neither deoxycholate (a common detergent used in Na^+,K^+-ATPase assays) nor alamethicin (a membrane-permeabilizing agent) had any effect on the total Na^+,K^+-ATPase activity in homogenates of *S. purpuratus* embryos, suggesting that inside-out and right-side-out vesicles are not a significant problem in assaying Na^+,K^+-ATPase activity in sea urchin embryos (Leong and Manahan, 1997). The protein content of the samples was determined by the Bradford assay with the modifications of Jaeckle and Manahan (1989).

Table 1

Comparison of nucleotide and deduced amino acid sequences for different α-subunit Na^+, K^+-ATPase

Species	GenBank accession number	Nucleotide identity (%)	Amino acid	
			Identity (%)	Similarity (%)
Drosophila	AF04494	69.8	73.3	89.2
Caenorhabditis	U18546	69.7	72.8	89.2
Xenopus	U49238	67.6	69.2	89.2
Artemia	X56650	65.2	72.3	89.7
Hydra	M75140	64.1	67.7	89.2

Identity and similarity to the *Strongylocentrotus purpuratus* cDNA open reading frame (clone #020) are presented as percentages determined from scoring by the GCG Wisconsin Users Group software program. Scores include only the terminal portion of the sea urchin gene's ORF: 588 base pairs (195 amino acid residues and stop codon).

Results

A partial *Strongylocentrotus purpuratus* cDNA clone (#020; Smith *et al.*, 1996) was characterized in this study and found to contain 1769 bp with the terminal portion of an ORF coding for 195 amino acids (588 bp with the stop codon). The remaining sequence (1181 bp) comprised a putative 3′ UTR domain. The clone's ORF was compared to other α-subunits of Na^+,K^+-ATPase, and the *S. purpuratus* nucelotide sequence ranged from 64% to 70% identity to these terminal ORF domains (Table 1). The deduced amino acid sequences of these organisms were aligned to the putative amino acid sequence of the *S. purpuratus* clone and evidenced a high degree of sequence conservation in this terminal domain (Table 2). When compared to the *S. purpuratus* sequence, the derived amino acid sequence was 68%–73% identical and 89%–90% similar (Table 1).

The terminal region of the ORF of known α-Na^+,K^+-ATPases is believed to contain several transmembrane domains; there is some debate over the exact number of these domains and the extra- *vs.* intracellular orientation of some of the intervening regions in α-Na^+,K^+-ATPase (Shull and Greeb, 1988; Takeyasu *et al.*, 1990; Blanco and Mercer, 1998). The hydropathy of the *S. purpuratus* sequence was estimated with Kyte-Doolittle scoring using a grouping of 11 amino acid residues (Fig. 1) and suggests a high probability of four transmembrane domains in the terminal portion of this ORF. Overlaying these domains on a structure detailed by Takeyasu *et al.* (1990) indicates that the region between the seventh and eighth transmembrane domains could have an extracellular localization. In the absence of crystallographic data, it is generally believed that most α-subunits of transmembrane ATPases (both Na^+ and Ca^{++}) are structurally similar, with 10 transmembrane do-

Table 2

Alignment of deduced amino acid sequence for the terminal 195 residues of the α-subunit of Na^+, K^+-ATPase in the sea urchin Strongylocentrotus purpuratus

```
          1                                                    
URCHIN    SDIMKRRPRD PQNDKLVNER LISVSYGQIG MIQRSAGFFA YFVIMGENGF
FLY       ADIMKRPPRD PFNDKLVNSR LISMAYGQIG MIQAAAGFFV YFVIMAENGF
SHRIMP    SDIMKRRPRN PVTDKLVNER LISLAYGQIG MIQASAGFFV YFVIMAECGF
NEMATODE  SDIMKRQPRD PIRDKLVNER LISLAYGQIG MIQASAGFFT YFWIMADNGF
FROG      SDIMKRQPRN PKTDKLVNER LISMAYGQIG MIQALGGFFT YFVILAENGF
HYDRA     SDIMKRHPRN PIRDKLVNER LISLAYGQIG MMQATAGFFT YFIILAENGF
            ***** *      ***** * ***  ***** * *   ***  ** *    **

          51                                                   
URCHIN    LPNDLIMLRS KWDDKAVLNV EDSYGQQWGF YQRKQLEYTC HTAFFASIVV
FLY       LPKKLFGIRK MWDSKAVNDL TDSYGQEWTY RDRKTLEYTC HTAFFISIVV
SHRIMP    LPWDLFGLRK HWDSRAVNDL TDSYGQEWTY DARKQLESSC HTAYFVSIVI
NEMATODE  MPWDLYQLRA QWDSRAYNNV LDSYGQEWTY ANRKILEYTC QTAYFVSIVV
FROG      LPWTLLGIRV NWDDRWTNDV EDSYGQQWTY EQRKIVEFTC HTSFFISIVV
HYDRA     LPSYLFGLRS QWDDMSNNNL LDSFGSEWTY FQRKEIELTC QTAFFTTIVV
           *          **          ** *  *     **  *  *  *  *  **

      101                                                   150
URCHIN    VQWADVIICK TRRNSLIHQG MNNWVLNFGL FFETALAAFL SYCPGLENGL
FLY       VQWADLIICK TRRNSIFQQG MRNWALNFGL VFETVLAAFL SYCPGMEKGL
SHRIMP    VQWADLIISK TRRNSVFQQG MRNNILNFAL VFETCLAAFL SYTPGMDKGL
NEMATODE  VQWADLIISK TRRNSLVQQG MSNWTLNFGL VFETALAWFM CYCPGLDNGL
FROG      VQWADLIICK TRRNSVFQQG MKNKILIFGL FEETALAAFL SYCPGMDVAL
HYDRA     VQWADLIISK TRRLSLFQQG MTNWFLNFGL FFETALAAFL QYTPGVNTGL
          ***** ** * *** *   ** * *    * *   ** ** *   * **    *

          151                                              195
URCHIN    RMYPLRIGWW FVAFPFSLLI FVYDECRRFI LRHNPGGWVE LETYY⌟
FLY       RMYPLKLVWW FPAIPFALAI FIYDETRRFY LRRNPGGWLE QETYY⌟
SHRIMP    RMYPLKINWW FPALPFSFLI FVYDEARKFI LRRNPGGWVE QETYY⌟
NEMATODE  RMYGLRFSWW FCALPFSILI FVYDEIRRFL IRRYPGGWVE RETYY⌟
FROG      RMYPLKPTWW FCAFPYSLII FIYDEVRKLI IRRSPGGWVE KESYY⌟
HYDRA     RLRPMNFTWW LPGLPFSLLI FVYDEIRRYL LRKNPGGWVE KETYY⌟
          *       **     *    * * ***
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The shaded blocks and bold lettering indicate the putative transmembrane domains identified by Kyte-Doolittle hydropathy scores in the sea urchin sequence (bold lettering; see Fig. 1). Genus names for the organisms and GenBank accession numbers for the sequences: urchin = *Strongylocentrotus* (this study), fly = *Drosophila* (AF04494), shrimp = *Artemia* (X56650), nematode = *Caenorhabditis* (U18546), frog = *Xenopus* (U49238), Hydra = *Hydra* (M75140); * = amino acid identity for all sequences; ⌟ = termination codon.

mains and a large extracellular loop between transmembrane domains 7 and 8 (Canfield and Levenson, 1993; Blanco and Mercer, 1998).

Northern blots using the 3′ UTR of clone #020 specifically recognized a 4.5 to 4.7 kb transcript under high stringency (Fig. 2). In another sea urchin, *Hemicentrotus pulcherrimus*, the full-length α-Na^+,K^+-ATPase cDNA has been cloned and has an mRNA transcript size of 4.6 kb (Mitsunaga-Nakatsubo *et al.*, 1992a). The α-Na^+,K^+-ATPase gene is differentially expressed during development in *S. purpuratus* (Fig. 2). The level of mRNA transcripts is low during early cleavage, then rises rapidly around gastrulation (at 25–36 h postfertilization; Fig. 3). After gastrulation, mRNA returns to a low level comparable to that initially found in the egg (Fig. 3). The rapid disappearance of the α-Na^+,K^+-ATPase transcript from the total RNA pool after gastrulation closely followed a first-order exponential decay model [$f(x) = 98.512\ e^{(-0.128x)}$; $r^2 = 0.988$; Fig. 3]. The decay constant of the regression is equivalent to a degradation rate of 7.8% h^{-1} of the transcript. At 83 h, α-subunit

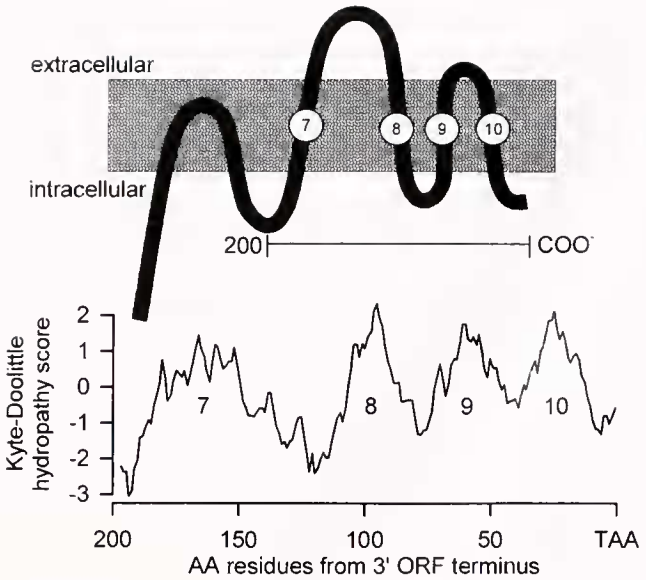

Figure 1. Secondary structure characterization for the last 195 amino acids of a putative α-Na^+,K^+-ATPase cDNA cloned from *Strongylocentrotus purpuratus*. The Kyte-Doolittle hydropathy score suggests the presence of four transmembrane domains, which match the structure of other α-Na^+,K^+-ATPase subunits (Blanco and Mercer, 1998).

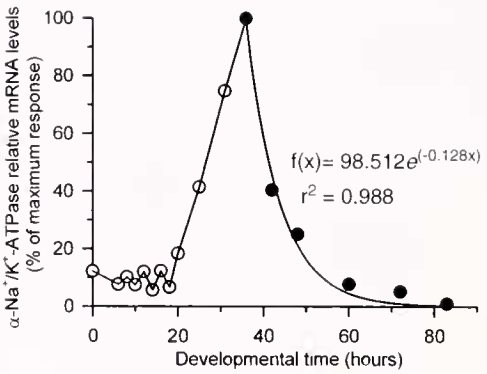

Figure 3. Relative transcript levels of the α-Na^+,K^+-ATPase cDNA during development in *Strongylocentrotus purpuratus* (quantified from Fig. 2). mRNA levels are presented relative to the maximal expression obtained at 36 h postfertilization. The rapid decline in mRNA abundance after 36 h fits a first-order exponential decay function (r^2 = 0.988; regression line plotted with shaded symbols).

transcripts were barely detectable under the conditions we used for Northern blots of total RNA.

The rapid increase in α-Na^+,K^+-ATPase mRNA transcripts during gastrulation in *S. purpuratus* was paralleled by a concomitant increase in the total activity of the sodium pump (Fig. 4). Activity levels were very low during early development in *S. purpuratus* and then increased after 20 h to a maximum level at the pluteus larval stage (Leong and Manahan, 1997). The rapid increase in activity between 20 and 40 h of development (Fig. 4) can be described by the exponential function [$f(x) = 1.167(1 + e^{[(x-x_0)/4.57]})^{-1}$; r^2 = 0.9664; maximum activity of 1.17 μmol P_i h^{-1} mg^{-1} protein]. The present study resolves the increase in enzyme activity at a finer time scale (*cf.* Leong and Manahan, 1997) and reveals the close coordination between α-subunit gene transcription and the assembly of functional sodium pumps in sea urchin embryos between fertilization and gastrulation.

Discussion

It has long been a general goal of physiological ecologists to identify a sensitive biochemical indicator of an animal's physiological state or metabolic activity—for example, the ratio of RNA to DNA (Westerman and Holt, 1994) or the glycolytic enzyme activities (Childress and Somero, 1990). For developmental stages with low biochemical contents, such assays are often not possible. Molecular biological techniques have the necessary sensitivity and potentially offer an alternative for assessing physiological state in larvae and small zooplankton. Because the sodium pump consumes such a large portion of cellular energy metabolism

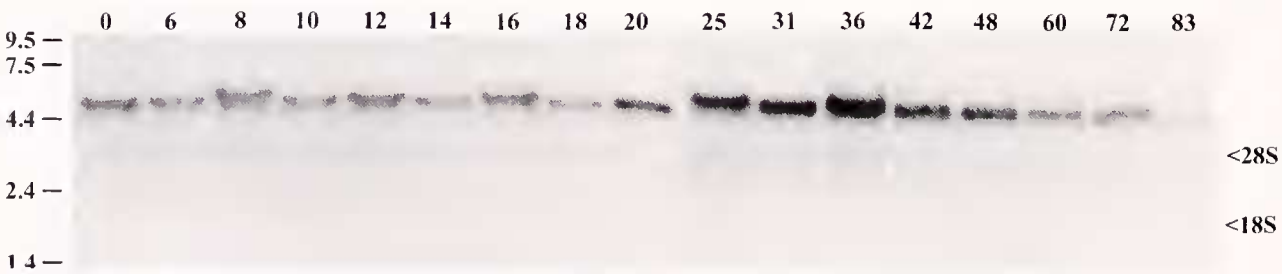

Figure 2. Northern blot hybridization of total RNA during development in *Strongylocentrotus purpuratus* using a radiolabeled probe from the 3′-untranslated domain of the α-Na^+,K^+-ATPase cDNA clone. RNA samples were collected at short time intervals during embryogenesis as shown by the hours post-fertilization at the top of each lane. Molecular size (kilobases) is indicated on the left; ribosomal RNA positions are indicated on the right. The probe recognizes a single transcript that is approximately 4.6 kb in size.

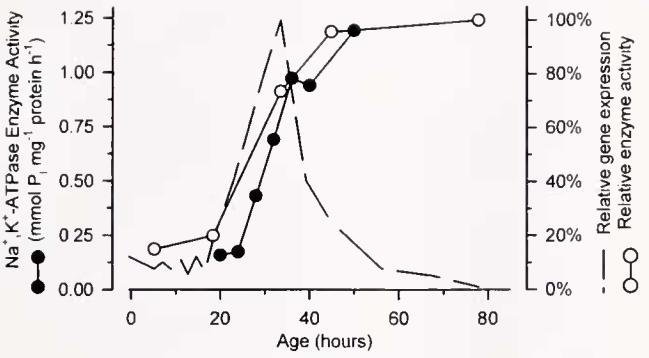

Figure 4. Total enzyme activity of Na^+,K^+-ATPase during gastrulation in *Strongylocentrotus purpuratus*. Protein-specific enzyme activity (closed circles) is plotted on the left axis; mRNA levels from Fig. 3 are overlaid (dashed line) to illustrate the temporal relationship between α-subunit gene expression and the appearance of functional sodium pump proteins. Data from Leong and Manahan (1997) are also plotted (open circles) to show the pattern of relative enzyme activities.

(*e.g.*, 40% in sea urchin larvae, Leong and Manahan 1997), it would seem to be a good candidate for such an assay, with the potential to provide sensitive information regarding rates of energy utilization in a single larva.

Several lines of evidence strongly support the conclusion that the partial cDNA clone (#020) in *Strongylocentrotus purpuratus* is the α-subunit of the sodium pump: (1) the putative amino acid sequences show a high similarity to those of other animals; (2) the 3′-UTR probe recognizes a 4.6-kb transcript, which is the full-length transcript size in other invertebrate species; (3) the ontogenetic increase in expression during gastrulation is similar to the expression pattern in another sea urchin (Mitsunaga-Nakatsubo *et al.*, 1992b); (4) total Na^+,K^+-ATPase enzyme activities show a concomitant increase as mRNA transcripts of clone #020 accumulate during gastrulation.

In the sea urchin *Hemicentrotus pulcherrimus*, the expression of the α-Na^+,K^+-ATPase gene increases rapidly during gastrulation (Mitsunaga-Nakatsubo *et al.*, 1992b). In *S. purpuratus*, the expression of the α-Na^+,K^+-ATPase gene evidences a similar pattern of ontogenetic regulation, with a sharp rise during gastrulation followed by a subsequent decline to much lower levels. In conjunction with the total Na^+,K^+-ATPase enzyme activity that is present during development (this study, Fig. 4; see also Leong and Manahan, 1997), temporal changes in both mRNA transcripts and protein activity indicate that the enzyme activity is low during early cleavage. At the point when an embryo approaches gastrulation, α-subunit gene transcription and subsequent mRNA translation increase greatly, producing a large increase in sodium pumps (Fig. 3), presumably as a necessary component of the physiological function of proliferating cells.

Once these pumps have been synthesized, mRNA transcripts for the α-Na^+,K^+-ATPase are rapidly lost. The decrease in mRNA levels over time fits a first-order exponential decay model (7.8% h^{-1}) so that by 83 h, transcription of the α-subunit gene was barely detectable (Fig. 2). At gastrulation, *S. purpuratus* appears to have synthesized most of the necessary sodium pumps. Total enzymatic activities show little increase after 50 h, further supporting this observation that the number of Na^+,K^+-ATPase ion pumps is set by the rapid transcription during gastrulation, and that once these transcripts are degraded, an early larva's sodium pump complement remains unchanged until further growth occurs, usually after feeding is initiated.

In vertebrates, the α-subunit Na^+,K^+-ATPase has several isoforms (Rossier *et al.*, 1987) that differ in many aspects, including sensitivity to proteases and cross-linking agents (Sweadner, 1979), electrophoretic mobility (Peterson *et al.*, 1982), and affinity for ouabain (Lytton *et al.*, 1985). In brine shrimp (*Artemia salina*), the α-Na^+,K^+-ATPase is present in two isoforms that are differentially expressed during early development (Peterson *et al.*, 1982). In the sea urchin *Hemicentrotus pulcherrimus*, two α-subunit isoforms are expressed during embryogenesis (Yamazaki *et al.* 1997). However, these two isoforms are encoded by a single gene and have identical sequences except for the 5′ leader sequences (Yamazaki *et al.*, 1997). If *S. purpuratus*, like *H. pulcherrimus*, has a similar isoform complement, then the cDNA probe we used for the present study (from the 3′-UTR) should hybridize to other α-subunit isoforms expressed during early development. Regardless of the mechanism, the disparity at 83 h postfertilization between the transcript measurements and the complement of active sodium pumps indicates the difficulty in isolating a single molecular factor to be used as an index for physiological rate processes.

The observation that Na^+,K^+-ATPase gene transcription and translation events are limited to a brief developmental period is intriguing. The sodium pump is considered to be a "housekeeping" protein. Consequently, for such an important physiological process, we would have expected the expression of a subunit gene to be constitutive and at a low level so that there would always be some subunit synthesis to replace any turnover in functional pump proteins. Such a continual level of replacement might have offered a sensitive assay for assessing the physiological state of individual larvae by providing a molecular index of the activity of one of the most energy-demanding cellular processes. However, this is not the case. The α-subunit expression is developmentally regulated so that gene expression is initiated rapidly at about 20 h, peaks at about 36 h, and is subsequently "turned-off." Such a temporal pattern of regulation highlights the difficulty of using molecular probes as simple indices of physiological state. Similar difficulties in the interpretation of physiological activity and expression have

been found for other specific housekeeping genes (*e.g.*, Weinstein *et al.*, 1992; Yang and Somero, 1996). For the multiple enzymes in metabolic pathways, the control mechanisms at the level of genes and proteins are even more complex (Hochachka *et al.*, 1998).

Ontogenetic changes in the metabolic rates of embryos have important consequences for subsequent survival because of the finite quantity of energy reserves in an egg. During development, metabolic rates increase in embryos as their cell numbers increase (Marsh *et al.*, 1999), and the activity of the sodium pump can consume a large fraction of total metabolism in some sea urchin embryos and larvae (Leong and Manahan, 1997, 1999). Understanding ontogenetic changes in sodium pump activities is important for assessing the metabolic energy costs of development. In the pluteus larval stage of *S. purpuratus* (at 83 h postfertilization), the *in vivo* sodium pump activity consumes 40% of total metabolism, with a potential reserve activity that could increase to a maximum of 77% of metabolism (Leong and Manahan, 1997). However, α-Na^+,K^+-ATPase gene expression is barely detectable at this point in larval development (Fig. 3). Consequently, molecular assays for expression of this gene would not be informative for assessing sodium pump activity as an index of a larva's physiological state. It is likely that during development and growth many physiological processes have functional rates of protein activity that are not strictly paralleled in time by the expression of their genetic components. A knowledge of the temporal relationship between gene and enzyme activities is critical to developing a molecular genetic index of physiological state in larval forms.

Acknowledgments

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